Nikon Training Notebook

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MSE Fee-For-Service Facility
Materials Science and Engineering
University of California, Riverside

May 20, 2021 (rev. 3.3)

Before you begin…

- ❑ Complete the required safety training modules on UC Learning
  - ❑ Laboratory Safety Orientation (Fundamentals) 2013
  - ❑ Hazardous Waste Management
  - ❑ Compressed Gas Safety
- ❑ Submit a copy of your Training Transcript to Lab Manager
- ❑ Review the MSE Policies and Regulations
- ❑ Fill out the MSE 150, 250, 309 FAU Authorization Form with PI signature
- ❑ Provide your ENGR username to Lab Manger to set up Faces account
- ❑ Arrange a time for training with Lab Manager
- ❑ Schedule your reservation on Faces for your training

Nikon Microscope Operation

I. Microscope Layout

II. Startup

III. EPI: Bright Field

IV. EPI: Dark Field

V. EPI: Polarization

VI. EPI: Differential Interference Contrast (DIC)

VII. DIA: Bright Field

VIII. Image Capture

IX. Cleanup

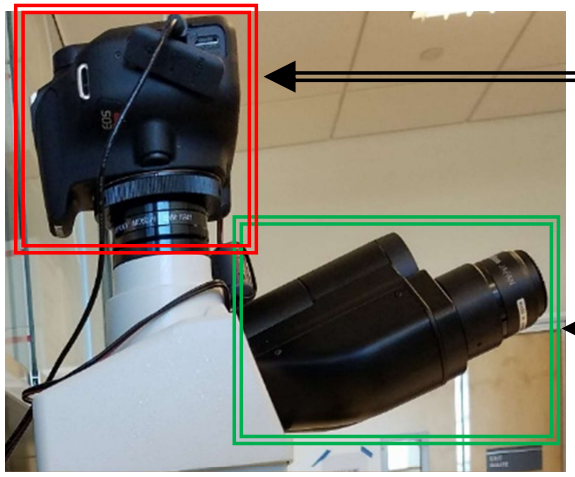
X. ImageJ

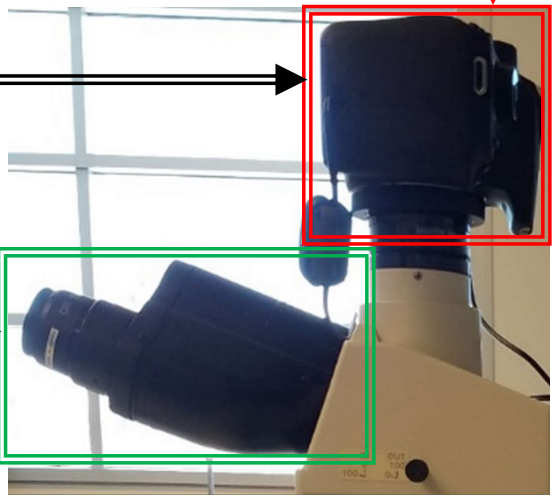
I. Microscope Layout – 1/4

Camera ON/OFF Switch

Camera Mode Selector:
M (default)

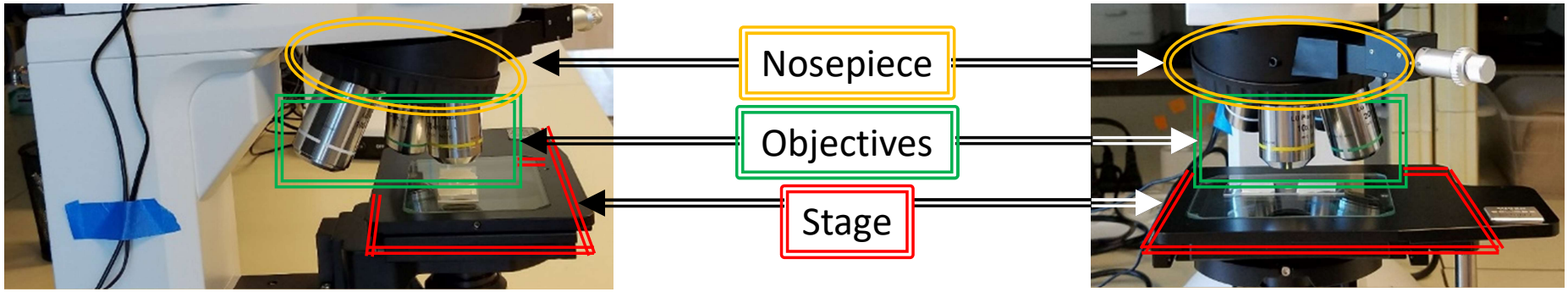
Camera

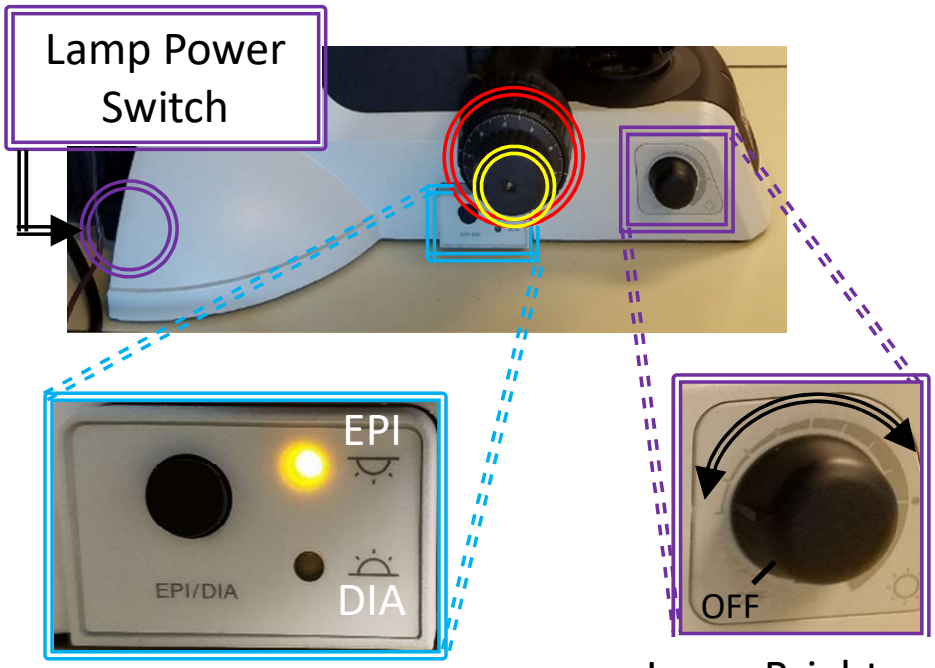




Binocular Eyepiece

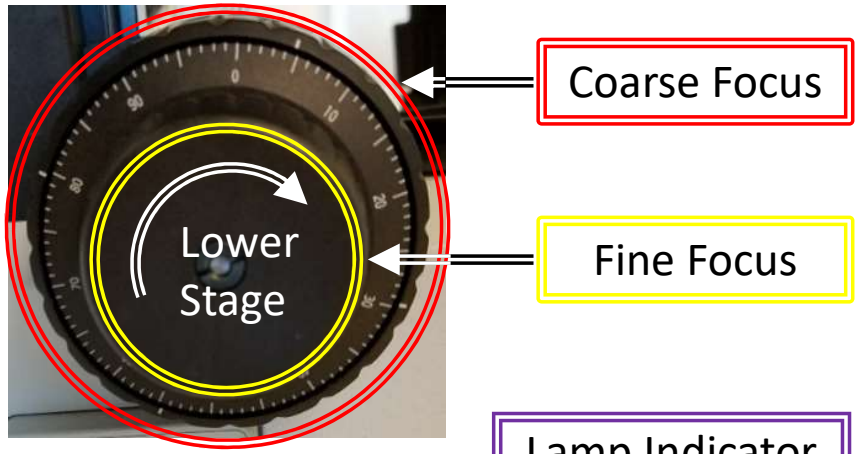
I. Microscope Layout – 2/4

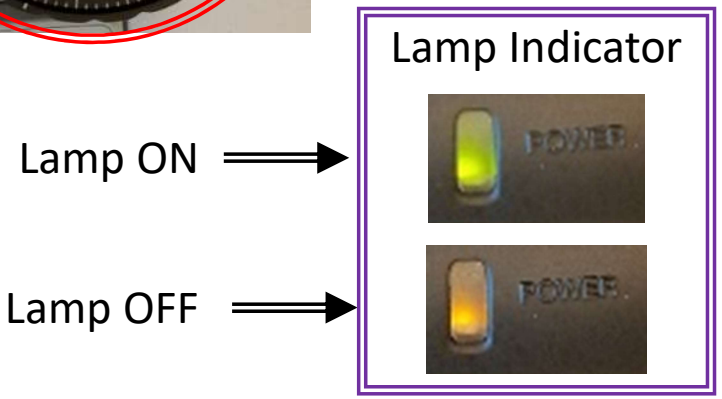




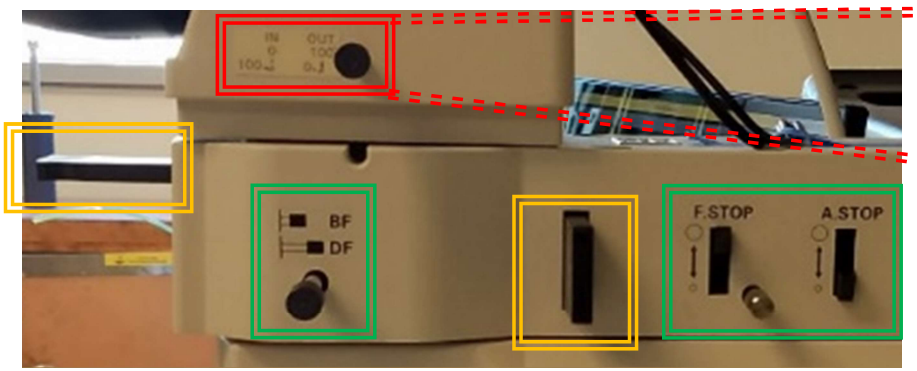
EPI/DIA Selector

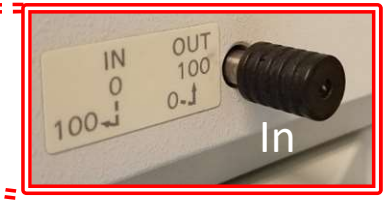
Lamp Brightness Knob

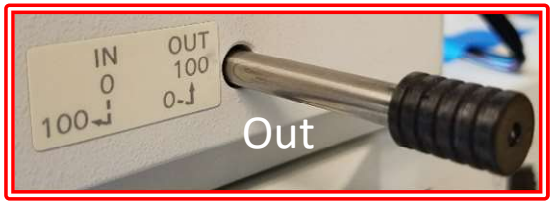




I. Microscope Layout – 3/4

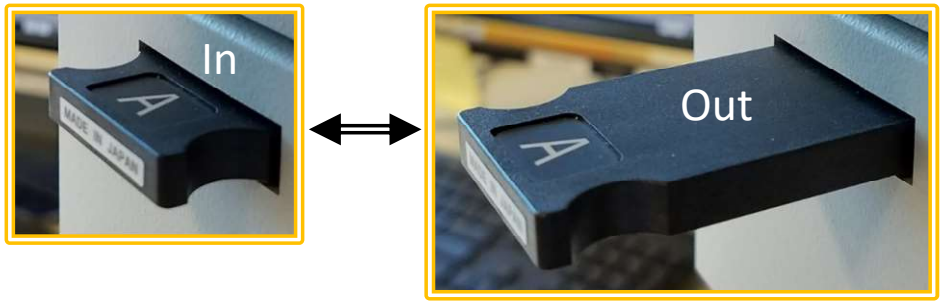




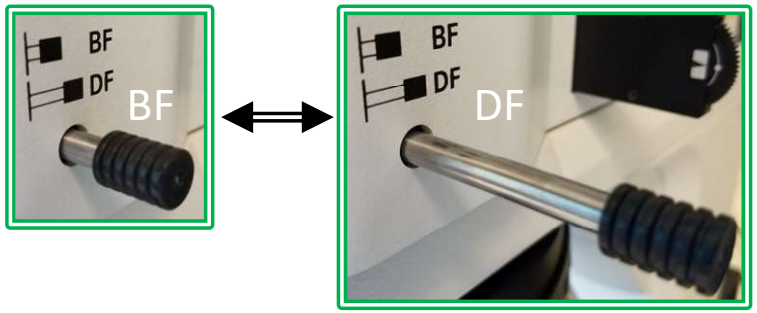


Optical Path Selector Lever

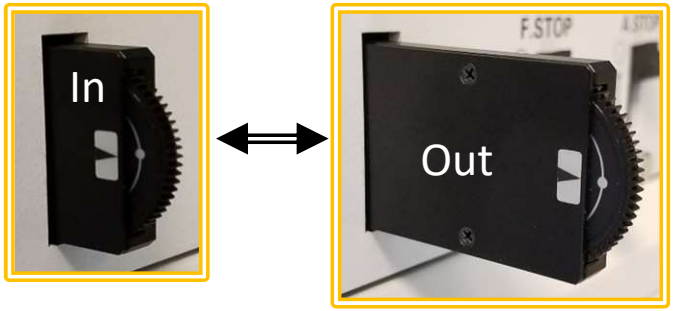
Analyzer Plate



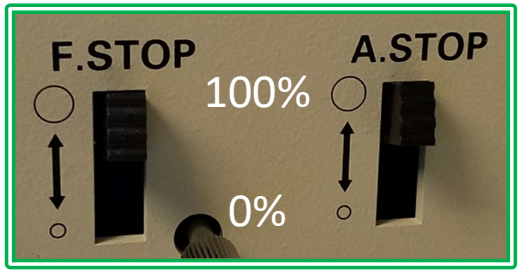
Bright field/Dark field Selector Lever



Polarizer Slider

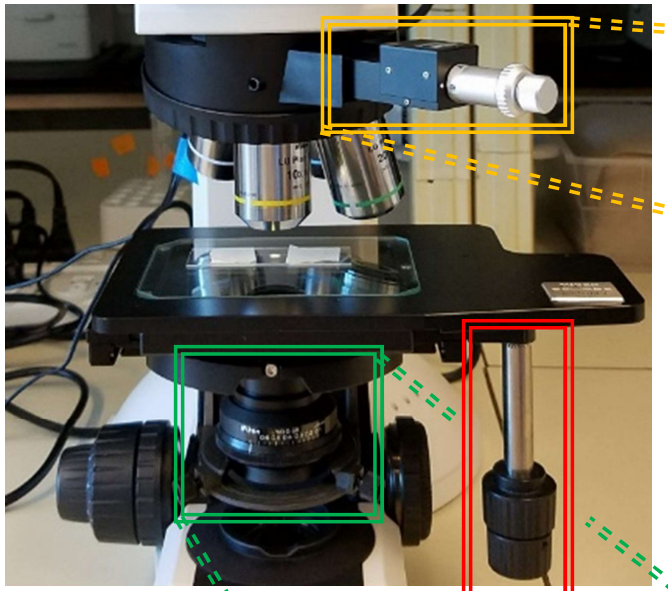


EPI Field Diaphragm Stop



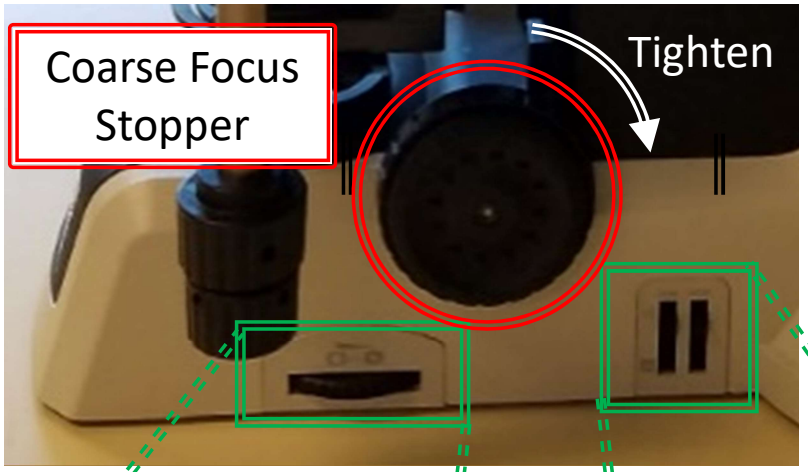
EPI Aperture Diaphragm Stop

I. Microscope Layout – 4/4



Stage Movement

DIC Prism Plate



DIA Condenser Focus Knob

DIA Condenser Aperture

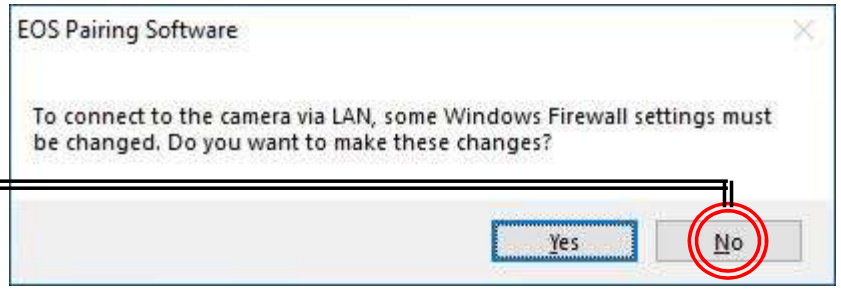
DIA Field Diaphragm Control

Filter Selector Switches

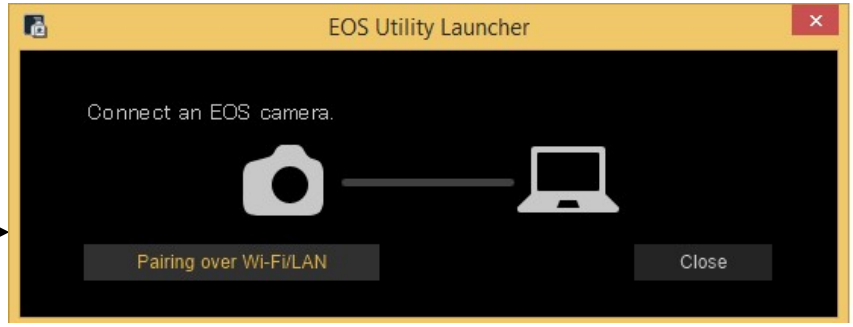
II. Startup – 1/5

1. Double-click on ***EOS Utility***

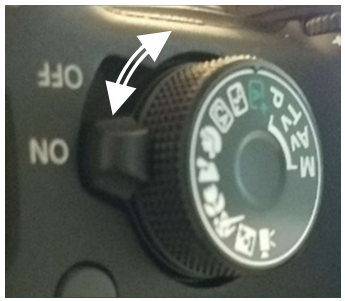
2. Click ***NO*** when asked to change Firewall settings



3. The EOS Utility Launcher may show that the camera is not connected to the computer



4. Toggle Camera ***ON/OFF*** switch to connect it to the computer – keep in ***ON*** position



5. Click on ***Camera settings/Remote shooting***

II. Startup – 2/5

6. Confirm the following ***Camera Settings*** are set:

Camera

M = Manual

Software (right click to change)

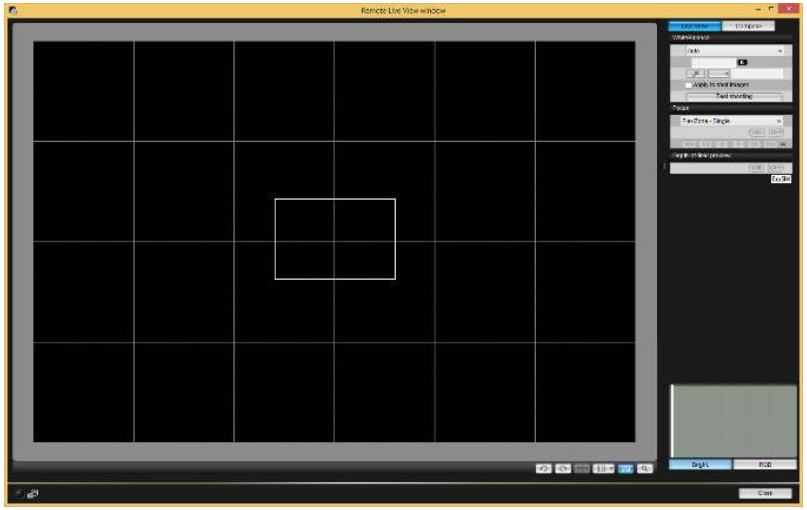
1/50 = Shutter Speed

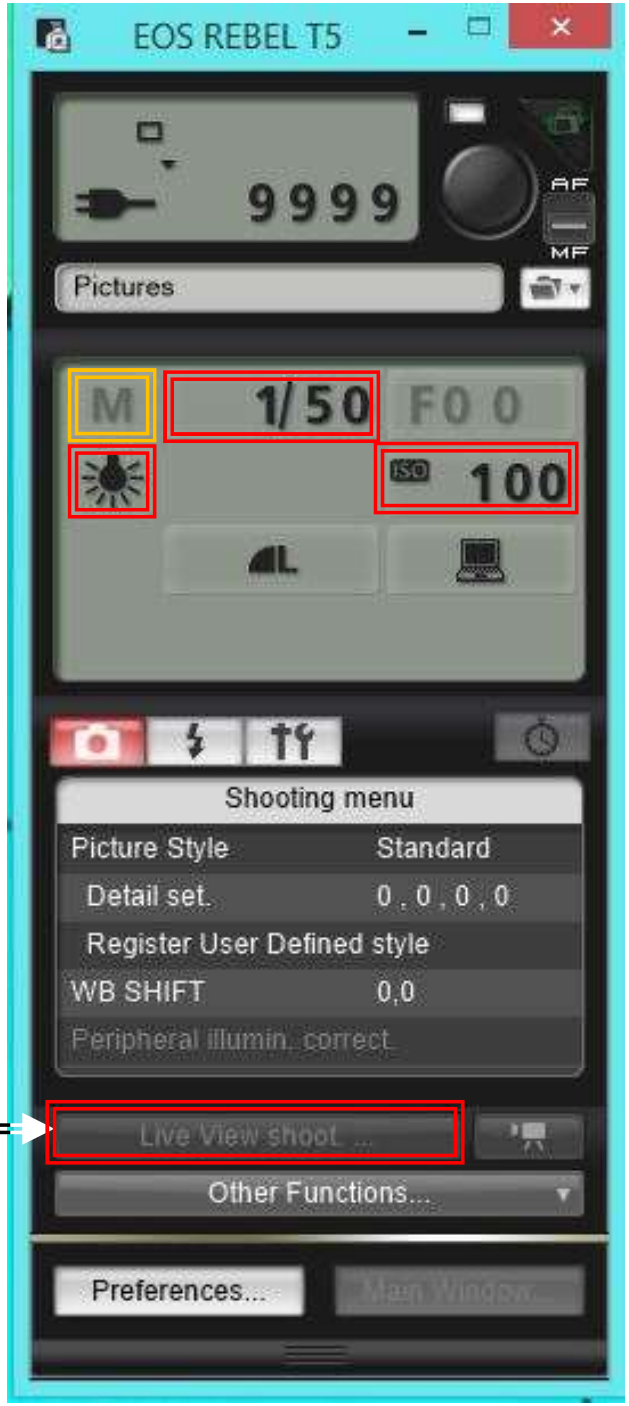
100 = ISO

Tungsten = Brightness

7. Click on ***Live View shoot***

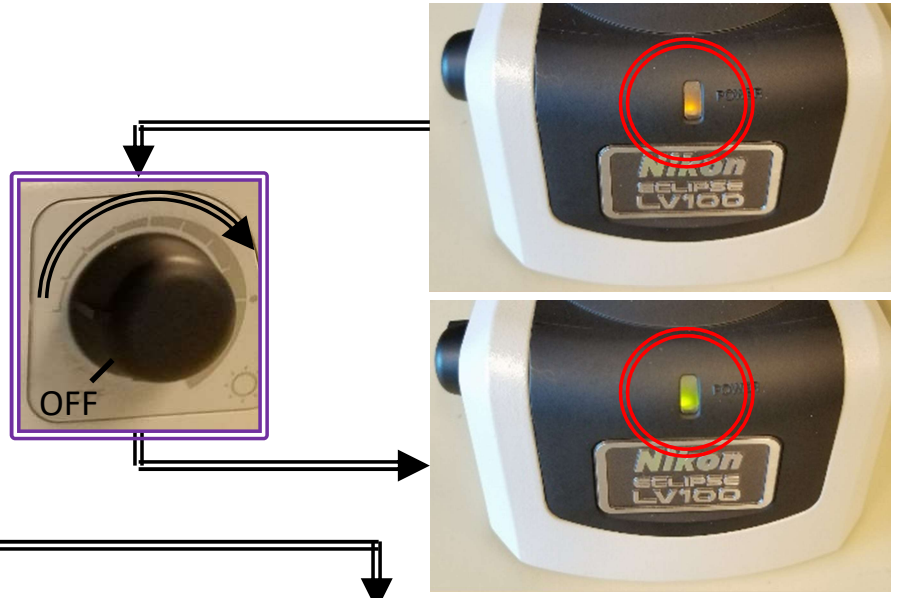
8. Remote Live View window will appear





II. Startup – 3/5

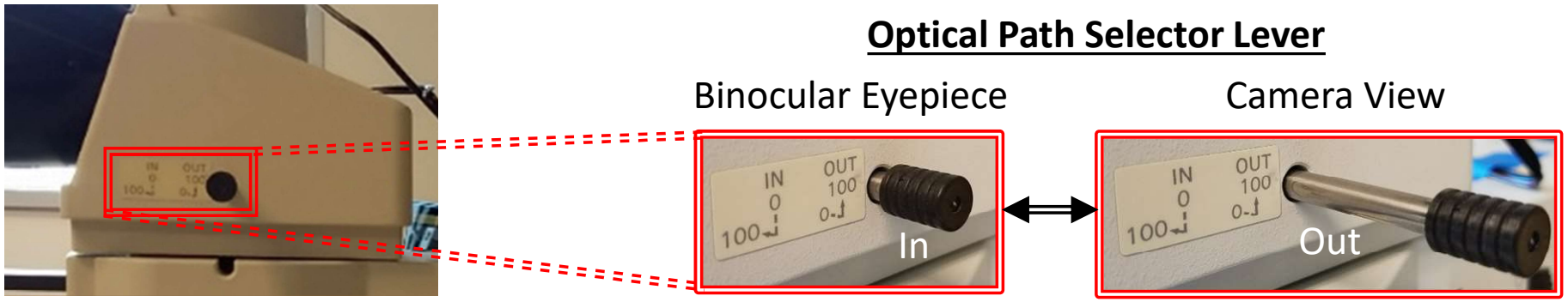
9. Rotate the ***Lamp Brightness Knob*** until the light indicator goes from ***orange (OFF)*** to ***green (ON)***



10. If light is missing, turn on ***Lamp Power Switch*** on back

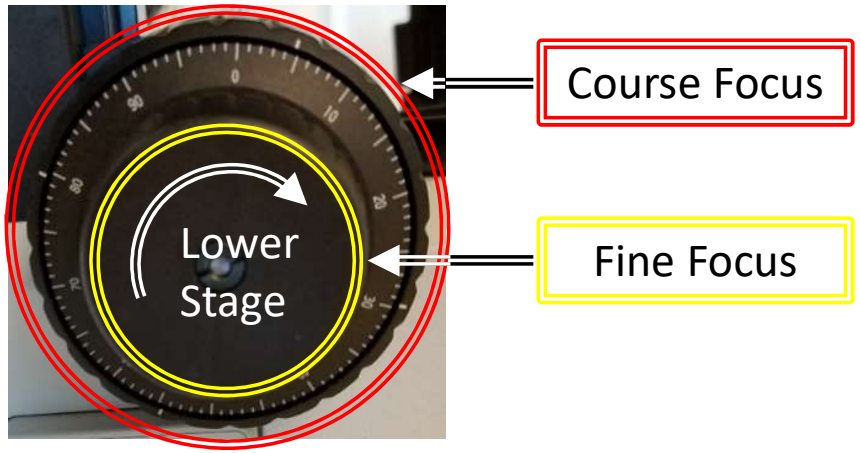
11. For ***Camera View***: Pull lever completely out

    For ***Binocular Eyepiece***: Push lever completely in



II. Startup – 4/5

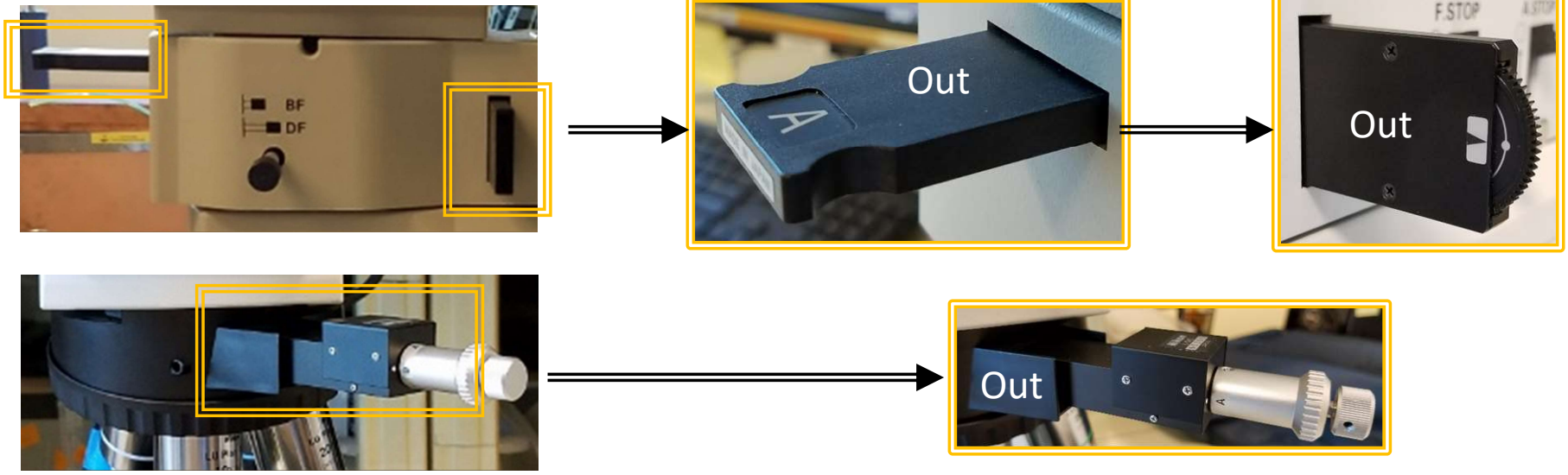
12. Lower stage first by turning ***Coarse Focus*** knob **TOWARD** you



13. Place sample on microscope stage

14. Rotate ***Nosepiece*** and start with the ***10X magnification*** first

15. Pull out ***Analyzer***, ***Polarizer*** and ***DIC Prism*** if inserted

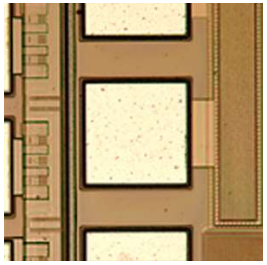


II. Startup – 5/5

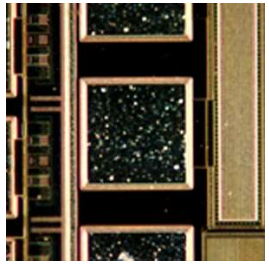
16. Identify which microscope mode you wish to use:

Episcopic Illumination ()

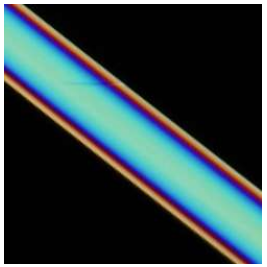
III. Bright field



IV. Dark field



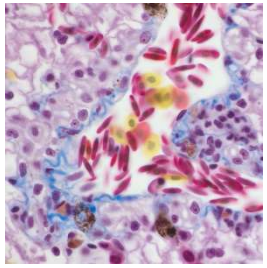
V. Polarization



VI. Differential Interference Contrast (DIC)

Diascopic Illumination()

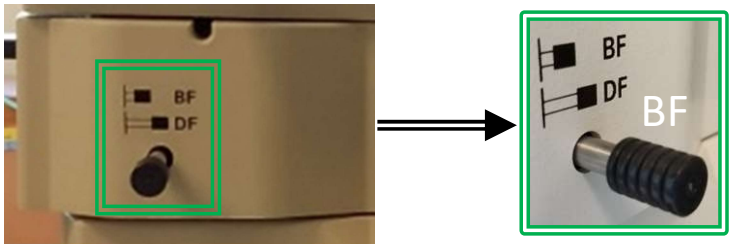
VII. Bright field



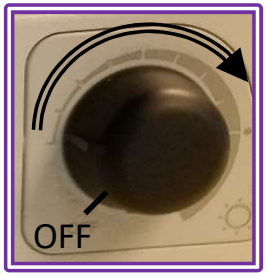
III. EPI: Bright Field – 1/3

1. Press the ***EPI/DIA*** selector and set to ***EPI***

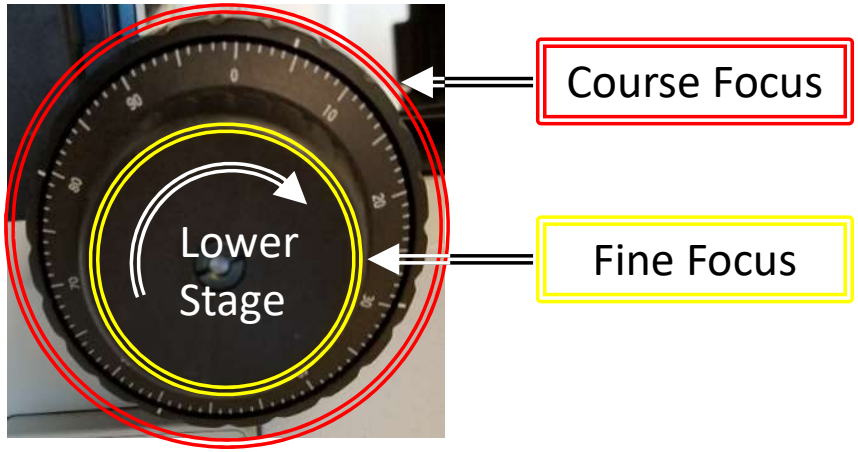
2. Push ***Bright/Dark Field*** selector lever to fully in ***BF*** position



3. Adjust the brightness with the ***Brightness Control*** as necessary

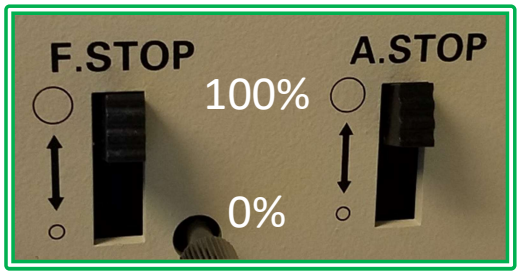


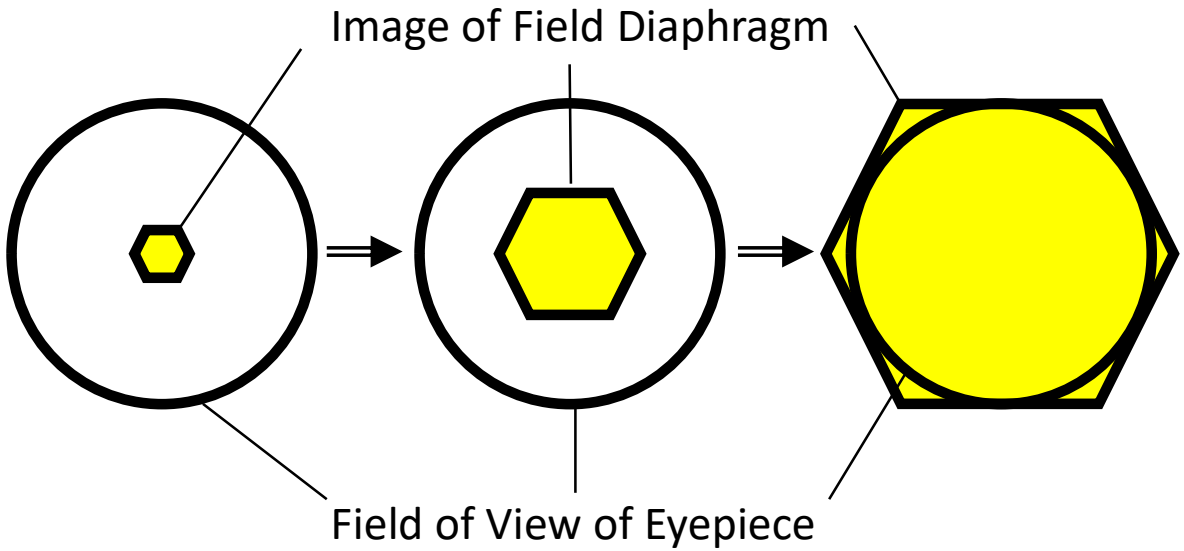
4. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs



III. EPI: Bright Field – 2/3

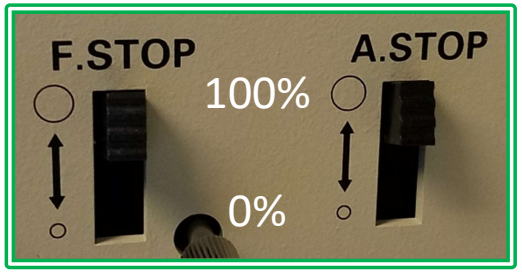
5. Adjust the ***F. STOP*** (field diaphragm) by sliding levers up and down until ***Image of Field Diaphragm*** circumscribes the ***Field of View***



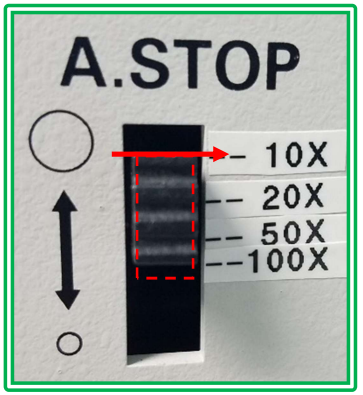


III. EPI: Bright Field – 3/3

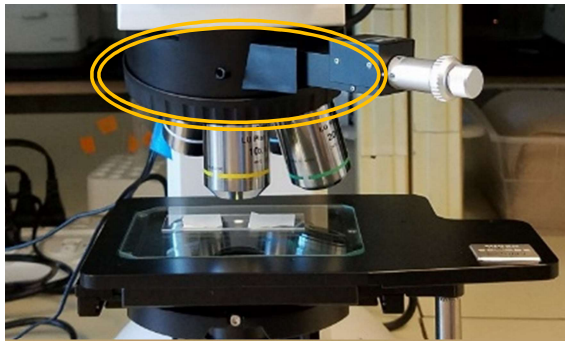
6. Adjust the ***A. STOP*** (aperture diaphragm) by sliding levers up and down to adjust depth of field



7. For each objective, recommended ***A. STOP*** position (top of lever) is shown on markings



8. Switch to higher magnification objectives if desired by rotating nosepiece

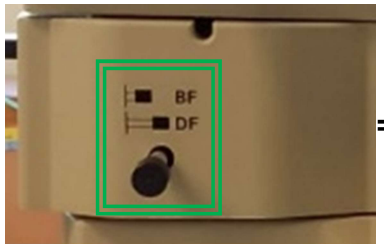


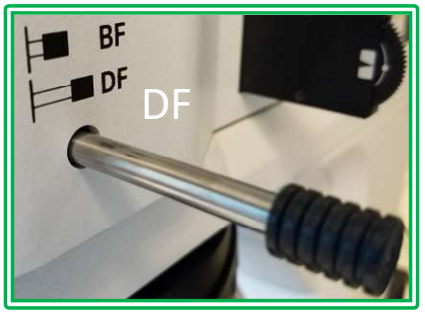
9. Repeat steps 3-9 until desired magnification and image quality is obtained
10. Go to ***Step VIII. Image Capture*** when ready to acquire image

IV. EPI: Dark Field – 1/2

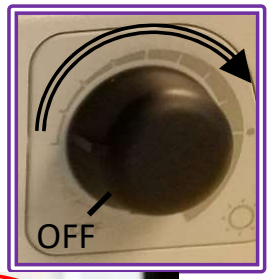
1. Press the ***EPI/DIA*** selector and set to ***EPI***

2. Pull ***Bright/Dark Field*** selector lever to fully out ***DF*** position

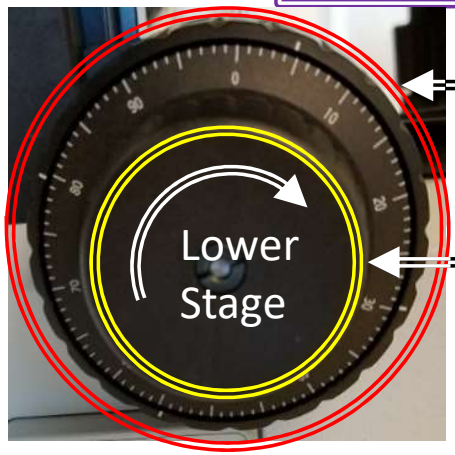




3. Adjust the brightness with the ***Brightness Control*** as necessary



4. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs

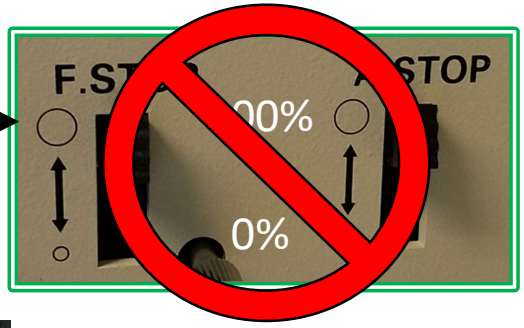


Course Focus

Fine Focus

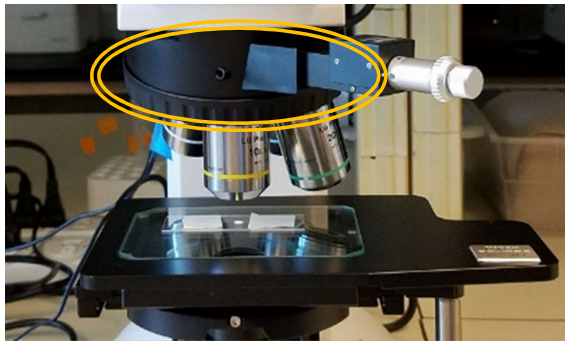
IV. EPI: Dark Field – 2/2

5. The ***F. STOP*** (field diaphragm) and ***A. STOP*** (aperture diaphragm) are automatically 100% open



   Levers will have ***NO*** affect

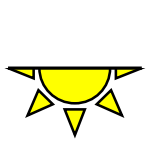
6. Switch to higher magnification objectives if desired by rotating nosepiece

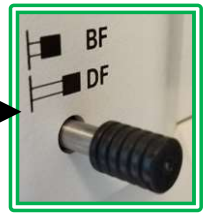


7. Repeat steps 3-6 until desired magnification and image quality is obtained

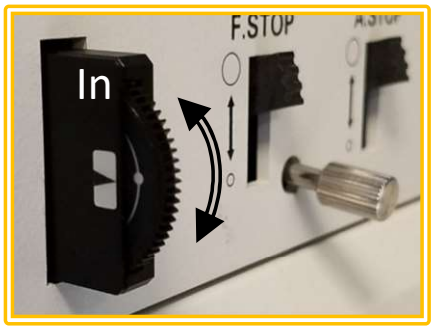
8. Go to ***Step VIII. Image Capture*** when ready to acquire image

V. EPI: Polarization – 1/2

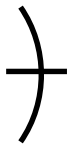
1. Press the ***EPI/DIA*** selector and set to ***EPI*** 

2. Adjust ***Bright/Dark Field*** selector lever to desired 

3. Push the ***Analyzer Plate*** in 

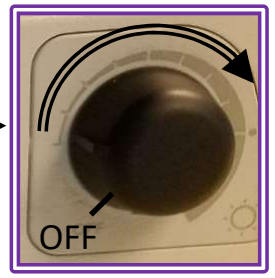
4. Push the ***Polarizer Slider*** in 

5. Rotate the polarizer to adjust the polarization from

lateral  to vertical 

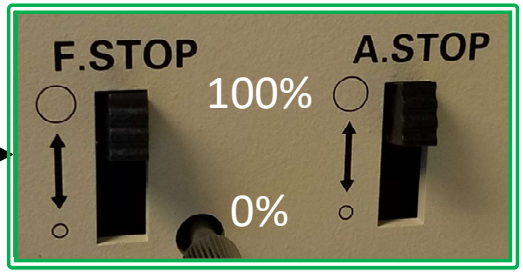
V. EPI: Polarization – 2/2

6. Adjust the brightness with the ***Brightness Control***

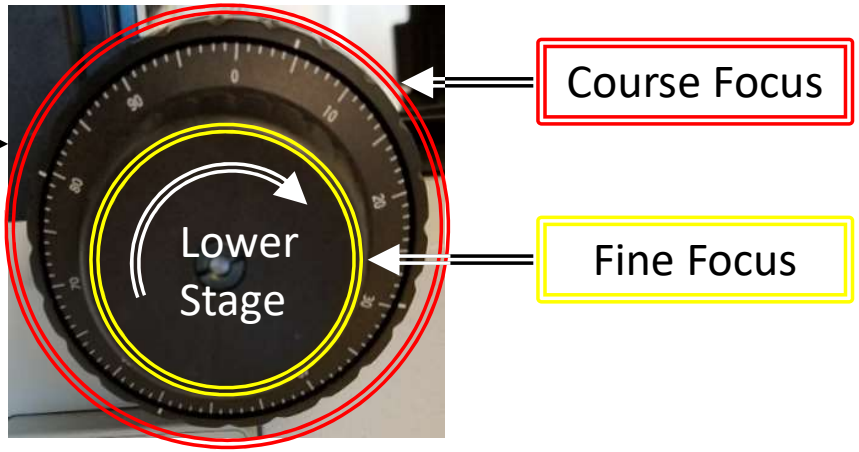


7. Adjust the ***F. STOP*** (field diaphragm) and ***A. STOP*** (aperture diaphragm) by sliding levers up and down from 100% open to 0% open

   Note: ***F. STOP*** and ***A. STOP*** levers will not work if in ***DF*** mode



8. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs
9. Switch to higher magnification objectives if desired by rotating nosepiece
10. Repeat steps 5-9 until desired magnification and image quality is obtained

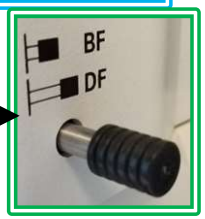


11. Go to ***Step VIII. Image Capture*** when ready to acquire image

VI. EPI: Differential Interference Contrast – 1/2

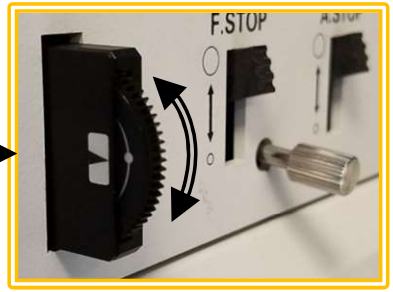
1. Press the ***EPI/DIA*** selector and set to ***EPI***

2. Adjust ***Bright/Dark Field*** selector lever to desired



3. Push the ***Analyzer Plate*** in

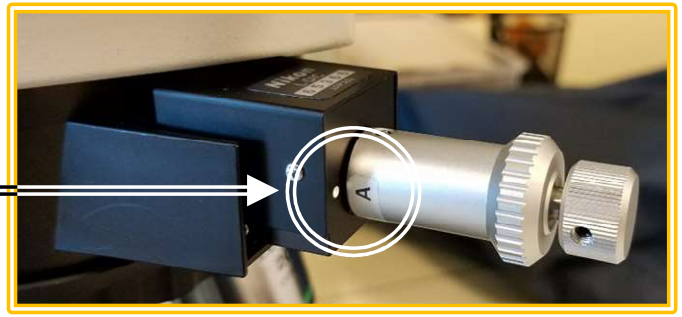
4. Push the ***Polarizer Slider*** in



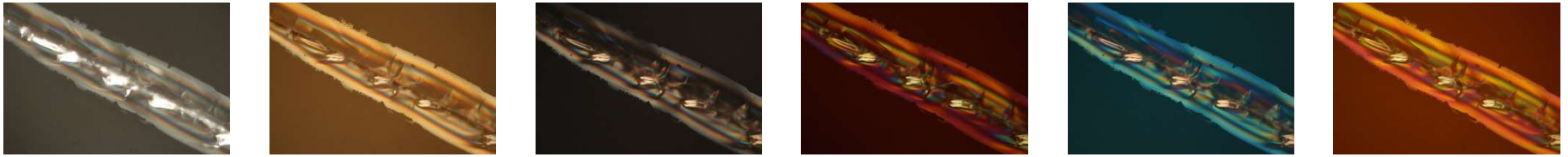
5. Rotate the polarizer to adjust the polarization from

lateral  to vertical 

6. Push the ***DIC Prism*** in and set to ***Position A***

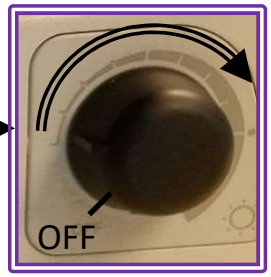


7. Rotate small knob to adjust contrast and color

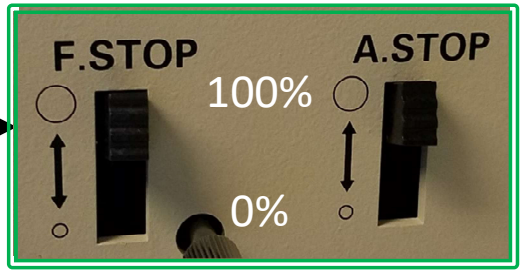


VI. EPI: Differential Interference Contrast – 2/2

8. Adjust the brightness with the ***Brightness Control***

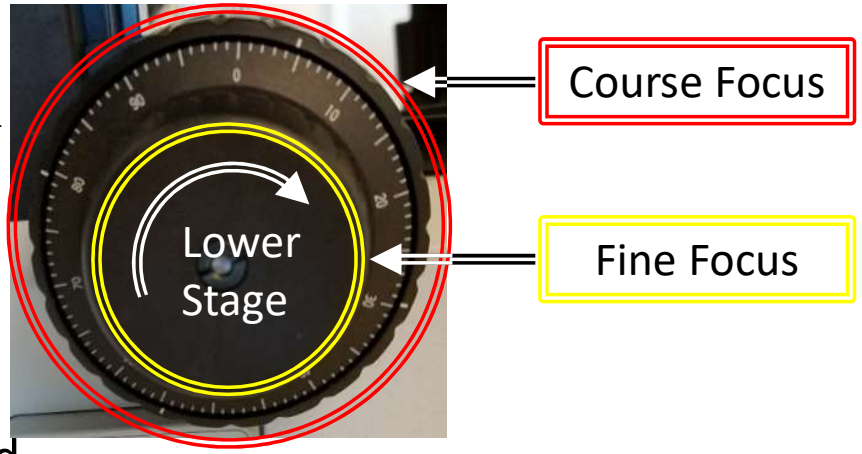


9. Adjust the ***F. STOP*** (field diaphragm) and ***A. STOP*** (aperture diaphragm) by sliding levers up and down from 100% open to 0% open



Note: ***F. STOP*** and ***A. STOP*** levers will not work if in ***DF*** mode

10. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs

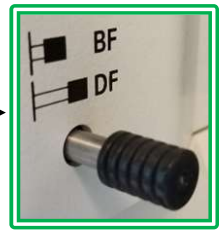


11. Switch to higher magnification objectives if desired by rotating nosepiece
12. Repeat steps 5-11 until desired magnification and image quality is obtained
13. Go to ***Step VIII. Image Capture*** when ready to acquire image

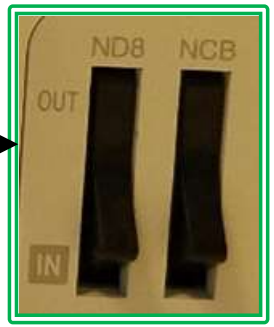
VII. DIA: Bright Field – 1/2

1. Press the ***EPI/DIA*** selector and set to ***DIA*** 

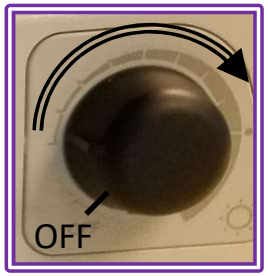
2. Push ***Bright/Dark Field*** selector lever to fully in ***BF*** position



3. Select ***NCB filter*** (balances color) if desired

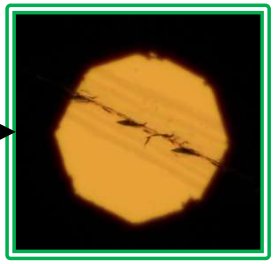


4. Adjust the brightness with the ***Brightness Control***



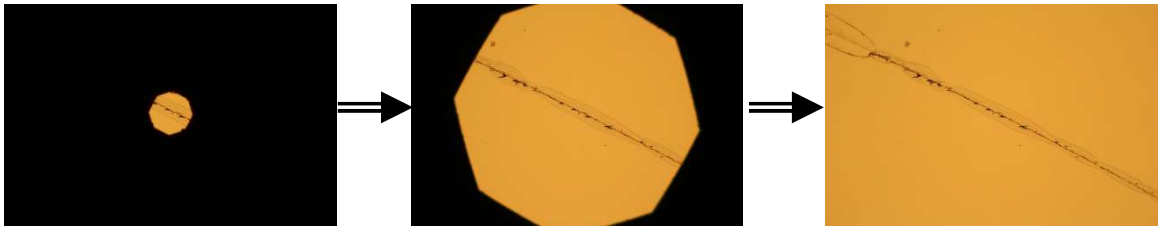
5. Adjust the ***Field Diaphragm Control*** to fully closed

6. Adjust the ***Condenser Height*** until the field diaphragm is focused



VII. DIA: Bright Field – 2/2

7. Center the field diaphragm by adjusting ***Centering Screws***
8. Open the ***Field Diaphragm Control*** until field diaphragm circumscribes the field of view



9. Focus on specimen by adjusting the ***Coarse/Fine Focus*** knobs
10. Adjust the ***Condenser Aperture*** to match ***Numerical Aperture*** for each objective:

10X = 0.3 20X = 0.45 50X = 0.8 100X = 0.9

11. Switch to higher magnification objectives if desired by rotating nosepiece
12. Repeat steps 3-11 until desired magnification and image quality is obtained
13. Go to ***Step VIII. Image Capture*** when ready to acquire image

VIII. Image Capture – 1/1

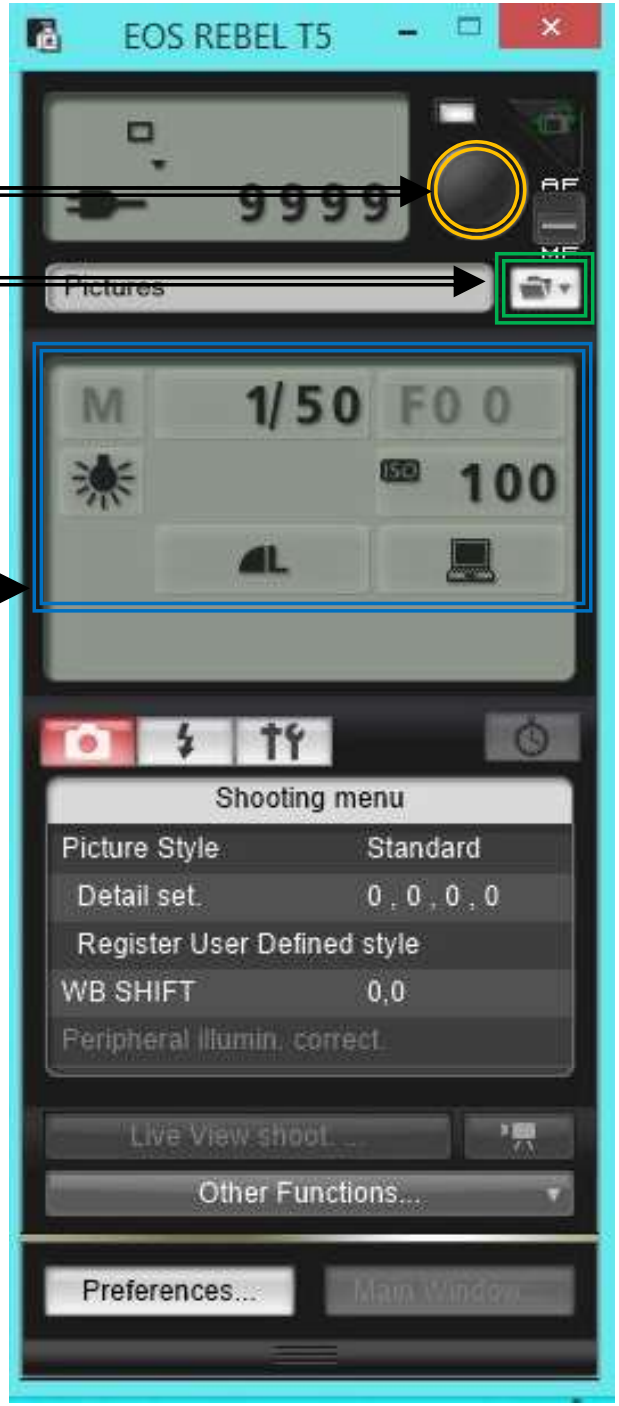
1. Click on the ***Folder*** icon and select desired folder to store saved pictures in

2. Recommend creating you own personal folder with sub-folders for each sample to help distinguish among them later

3. It is important to record the objective used for ***EACH*** image taken (necessary for scale)

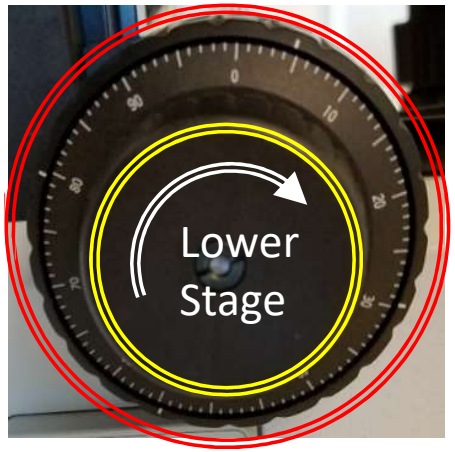
4. Review ***Camera Settings***

5. Click on the ***Shutter Button*** to acquire image



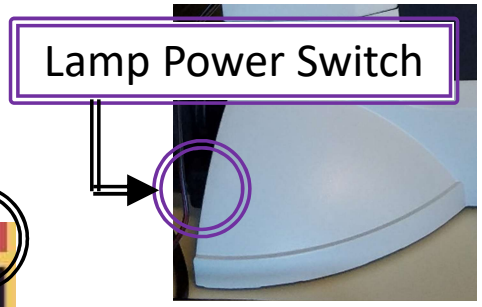
IX. Cleanup – 1/1

1. Lower the stage away from the objectives by rotating the ***Coarse Focus*** knob **TOWARD** you



2. Rotate nosepiece and place the ***10x Objective*** into position

3. Turn off ***Lamp Power Switch*** at the back of the microscope



4. Turn ***OFF*** the control software

5. ***Log Out*** of your ENGR account

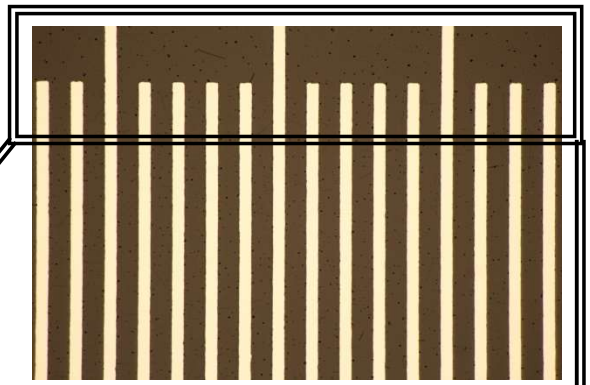
6. Clean up and dispose of any consumables used and return any tools back to its respective containers or bins

7. Confirm that the microscope is turned **OFF** again (**NO LIGHT!**), then place cover over microscope

X. ImageJ – 1/3

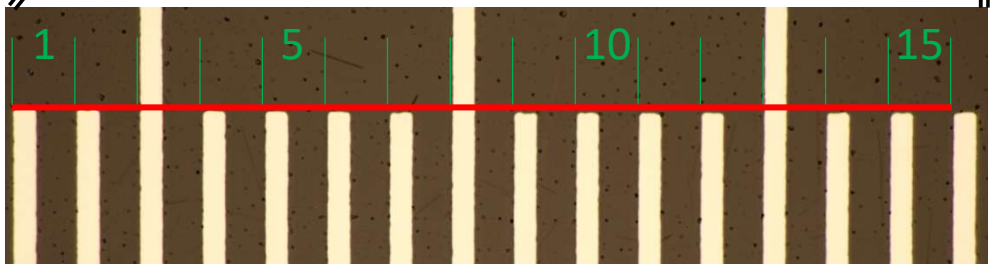
1. Double-click on ***ImageJ*** icon

2. Click ***File > Open***
3. Locate the ***Scale Bar Images*** folder
4. Select the ***Magnification*** of the image you wish to measure (e.g. 100X) and ***Open***
5. Click the ***Segment Tool*** and select ***Straight Line***



6. Draw a line that contains the maximum number of tick marks

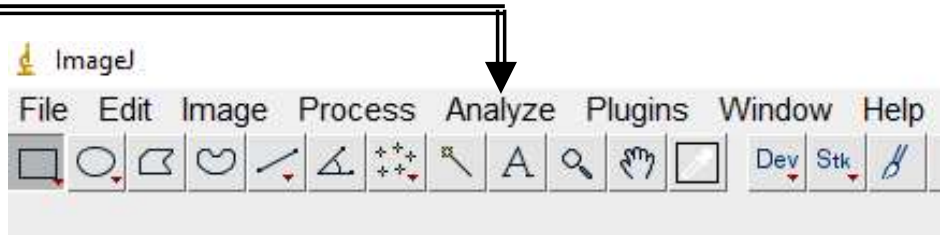
   Note: It matters where you start and end the line!



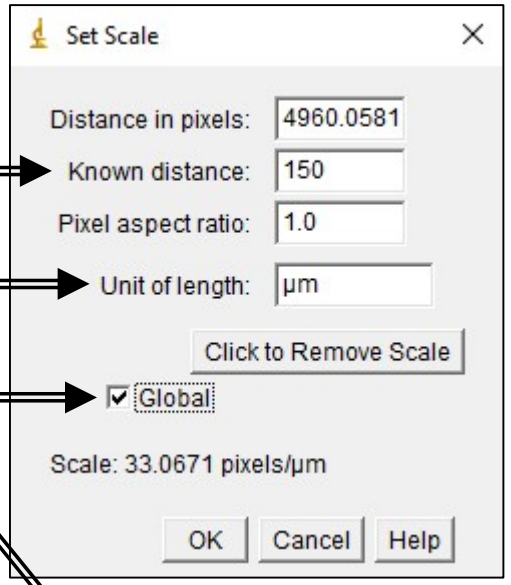
7. Count the number of tick marks contained (e.g. 15)
8. Each division is 0.01 mm (or 10 μm)

X. ImageJ – 2/3

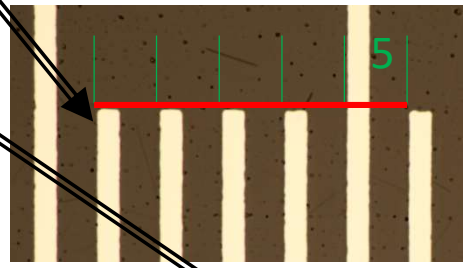
9. Click ***Analyze > Set Scale***



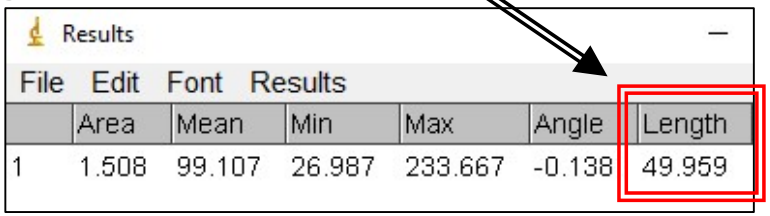
10. Enter the ***Known Distance*** (e.g. 150 μm) based on the number of tick marks and each division = 0.01 mm (or 10 μm)
11. Enter the ***Unit of Length*** to desired unit (e.g. mm)
12. Check ***Global*** to set scale for all images
13. Confirm your scale by drawing a new ***Straight Line***
14. Click ***Analyze > Measure*** and check value



If incorrect, repeat steps 5 – 13

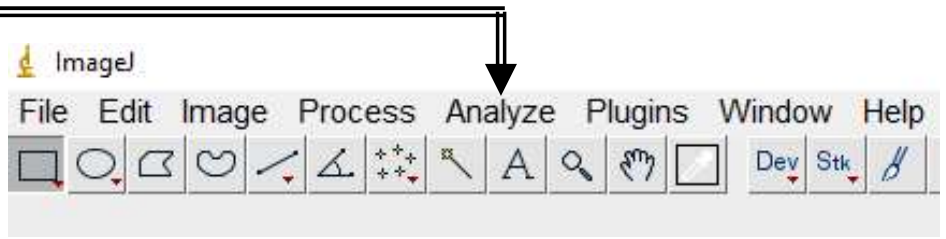


15. Click ***File > Open*** and select your image(s) of interest
16. Draw ***Straight Lines*** and click ***Analyze > Measure***
17. Repeat steps 4 – 16 for other ***Magnifications***



X. ImageJ – 3/3

9. Click ***Analyze > Tools > Scale Bar***



10. Enter ***Width in um*** (e.g. 50 µm)
    based on the length of scale bar desired

11. Enter ***Height in pixels*** for desired scale bar thickness

12. Enter ***Font size*** for desired text size

13. Identify ***Color*** of the scale bar

14. Identify ***Background*** color (if desired)

15. Identify ***Location*** where ***Scale Bar*** to be placed